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The effect of host age on mosquito attraction, feeding, and fecundity.

Dennis Albert LaPointe
University of Massachusetts Amherst

THE EFFECT OF HOST AGE ON MOSQUITO ATTRACTION, FEEDING, AND FECUNDITY

A Thesis Presented

By

DENNIS ALBERT LAPOINTE

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Approved as to style and content by:

(Dr. John D. Edman), Chairperson of Committee

(Dr. Ronald J. Prokopy), Member

(Dr. John G. Stoffolano, Jr.), Member

(Dr. John D. Edman), Department Head
Entomology

DEDICATION

To my parents, Claire A. and Albert D. LaPointe, for making my education possible.

ACKNOWLEDGEMENT

I would sincerely like to thank my advisor, Dr. John D. Edman, for the support, advice and patience he has directed toward me throughout this study. I would also like to thank the other members of my committee, Dr. Ron Prokopy and Dr. John G. Stoffolano, Jr., for their advice and criticism of this manuscript.

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Finally, I would especially like to thank my sister Carol for her support and typing, Ken and Annie for their kindness, and Pam for all the things that need not be said.

ABSTRACT

Attraction of Aedes aegypti (L.) to adult and maturing young of four small mammal species (laboratory rat, domestic rabbit, Eastern cottontail (Sylvilagus floridanus (Allen)) and opossum (Didelphis marsupialis L.)) was evaluated using both convective air flow olfactometers and a flight-tunnel olfactometer modified with a choice chamber. The importance of single attractive stimuli in influencing differential attraction was investigated using similar techniques. Observations of mosquito feeding on adult and young hosts and the defensive behaviors of hosts were made. Blood feeding experiments on adult and young rodents were also conducted to determine if fecundity was affected by host age.

Three of the four young hosts tested were found attractive throughout their development. Opossums in the marsupium were not attractive when removed and tested, but were not found to be repellent, either. Attraction to young hosts increased with body development and critical weights at which attraction to adult and young was comparable were found for each host. Litters with a combined weight greater than the critical weight were as attractive as, or more attractive than the adults. Attraction was strongly correlated to host weight, but

no single weight-associated attractive stimulus (heat, moisture, CO_2, odors) was found to be critical in effecting differential attraction.

Young hosts displayed fewer and less effective defensive behaviors than corresponding adults, and mosquitoes readily engorged on all young hosts. Fecundity was not affected by host age but was reduced, in later feedings, when rapidly repeated feedings were made on suckling mice.

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LITERATURE REVIEW

A Note of Terminology

In behavioral studies involving insect orietation, terminology has always been confusing. The literature concerning mosquito host-seeking behavior spans seventy-five years and the terms 'attractant', 'repellent', 'stimulators', and 'activators' have often been used carelessly. In this study these behavioral concepts are defined according to Dethier *et al*. (1960).

Host-Seeking Behavior

Mosquito host-seeking behavior has been a prime interest of researchers for many years and a great bulk of literature has accumulated concerning this subject. Most early research was performed with the yellow fever mosquito *Aedes aegypt*(L.) and is reviewed in Christopher's (1960) monograph on this species. More recent reviews are by Clements (1963), Brown (1966), Hocking (1971) and Khan (1977).

Unfortunately, this great mass of work has yet to reveal a unified theory of host-seeking which is generally accepted (Hocking, 1971). Conclusions of contemporary research are striking similar to — and often as vague as —

work performed in the early 1920's. Among contemporary researchers there are many conflicting opinions with two distinct schools of thought emerging concerning the primary stimulus for mosquito attraction.

Wright and his co-workers (Kellogg and Wright, 1962; Daykin et al., 1965; Wright, 1975) suggest that mosquito attraction is basically a function of host-derived heat and moisture and the subsequent convective currents. Various other researchers (Price et al., 1979; Omer, 1979; Acree, 1967) disagree with this theory and suggest that the major stimuli for attraction are specific chemicals emanating from the host. Although the primary stimulus of attraction is still in debate, most researchers agree on the basic physical and chemical factors important in host-seeking. Aside from the visual stimuli – which are not considered in this study – these factors are heat, moisture, CO_2 and host odors. The following sections will deal with each factor separately.

Heat. Early studies by Howlett (1910) and Marchand (1918) demonstrated the importance of temperature in mosquito attraction. Although Howlett suggested the connection with convective currents, Peterson and Brown (1951) were first to show that attraction due to heat is based on convective heat and not radiant heat. The importance of convection currents and temperature gradients has been

repeatly stressed by some researchers (Daykin et al., 1965; Wright, 1975), but others have only considered these factors important in close range attraction to the host (Khan et al., 1966; Kalmus and Hocking, 1960). Also, there are conflicting views on the attractiveness of dry heat (Peterson and Brown, 1951; Wright and Kellogg, 1962), but it is generally accepted that moist heat is very attractive (Khan and Maibach, 1966; Bar-Zeev et al., 1977; Price et al., 1979).

Moisture. Brown (1966) reported moisture to be the single most important factor in close range host-seeking behavior. Generally, early studies agree on the importance of moisture (Crumb, 1922; Parker, 1948; Brown, 1951), although others have reported indifference or avoidence of low and high humidities (Rudolfs, 1922; Muirhead-Thomson, 1938; Smart and Brown, 1956; Platt et al., 1957; Bar-Zeev et al., 1977). More recently the role of moisture alone in mosquito attraction appears to be negligible and responses to moisture in earlier studies are thought to be due to varying physiological states (i.e. water stress) (Bar-Zeev, 1960; Price et al., 1979).

Carbon Dioxide. Carbon dioxide was first identified by Rudolfs (1922) as a stimulator of mosquito behavior. Since then its role in host-seeking behavior has become a

controversial issue. Various investigators have confirmed CO_2's role as a stimulator (Kellogg and Wright, 1962; Khan and Maibach, 1966; Omer, 1979), while others have suggested it to be an attractant as well. Working with Aedes aegypti (L.), Roth and Willis (1952) demonstrated CO_2 repellency in a small olfactometer and attractancy in a larger olfactometer. Similarly, Brown et al. (1951) and Bar-Zeev et al. (1977) concluded that CO_2 was attractive to Aedes aegypti(L.), while Mayer and James (1970) suggested that CO_2 only synergized attraction for host odors. Many species other than Ae. aegypti have demonstrated attraction to CO_2 in both laboratory and field studies (Reeves, 1953; Snow, 1970; Gillies and Wilkes, 1969, 1972; Edman, 1979; Omer, 1979). An excellent review on this subject has been written by Gillies (1980).

Odor. Recent studies with cold trapping host emanations (McIver, 1968; Price et al., 1979) have confirmed the earlier work (Clements, 1963) which demonstrated that host odors are attractive. Whole blood, sweat and urine have all been tested and found attractive to some degree (Parker 1948; Thompson and Brown, 1955; Burgess and Brown, 1957; Roessler, 1961; Khan et al., 1969), but the search for specific attractive chemicals has led

researchers to analyze various volatile components of human skin emanations. Although estrogens and amino acids such as lysine and alanine (Brown and Carmichael, 1969; Roessler and Brown, 1964; Bos and Larman, 1975) have been shown to be attractive, the most powerful attractant isolated to date has been L-lactic acid (Acree et al., 1968; Carlson et al., 1978). In no cases have reconstituted odors or isolated chemicals proved as attractive as the live host.

Mosquito Repellency

Undoubtedly, more research has been directed at mosquito repulsion than attraction, and the number of compounds tested for repellent qualities is enormous. From this research two classes of repellents have been defined (Sarkaria and Brown, 1951). Vapor or olfactory repellents are volatile and function at a distance away from their source. Contact or gustatory repellents are relatively non-volatile and for this reason must be contacted directly. Although most mosquito repellents are either synthetic or derived from plants, Skinner et al. (1965, 1967) have observed repellency to naturally occurring human skin-surface lipids. Research concerning the chemical and physical parameters of repellency has been reviewed by Garson and Winnike (1968).

Theories on repellents' mode of action are speculative and will probably remain so until a better understanding of attraction has been reached (Wright, 1956; Gouck and Bowman, 1959; Hocking and Khan, 1966). Wright (1975), dogmatically convinced of the attractive importance of moisture, postulated that repellents function by blocking hydroreceptors. Recently it has been found that the repellent DEET (N_1N-diethyl-m-toluomide) inhibits the lactic acid receptors of Aedes aegypti (L.) (Davis and Sokolove, 1976). This fact and the findings demonstrating the relative unimportance of moisture in attraction (Bar-Zeev et al., 1977; Price et al., 1979) should put a damper on Wright's argument.

The Use of Olfactometers

Most of the research on mosquito attraction and repulsion has been performed in small laboratory olfactometers. The design of these devices vary from the simple single-port passive air flow models (Khan et al., 1966; Feinsod and Spielman, 1979) to complex multiple-port forced-air apparatuses (Mayer and James, 1969; Price et al., 1979). Forced-air models may have either a vertical (Daykin and Kellogg, 1965; Bar-Zeev et al., 1977) or horizontal (Gouck and Schreck, 1965, Mayer and James, 1969; Price et al., 1979) orientation and

almost all have equipment to regulate temperature, humidity and air-borne chemicals. Multiple ports for treated air provide choice situations, and mosquitoes are generally trapped at these ports.

In an attempt to simulate the anemotaxic flight of host-seeking mosquitoes, Mayer and James (1969) constructed an olfactometer based on wind tunnel design. Their olfactometer allowed for testing mosquito orientation to both upwind and downwind attractive sources, as well as distinguishing between attraction and stimulation behaviors (Mayer and James, 1970; Omer, 1979).

Feinsod and Spielman (1979) suggested that behavioral complexities associated with forced air should be avoided by use of passive air flow systems. They also pointed out the unnaturalness of choice situations and difficulties of maintaining separate air streams from multiple ports. Their passive air flow olfactometer is simple in design and assures a quick and accurate identification of host-seeking mosquitoes before host contact.

Host Preference and Mosquito Attraction

Host feeding patterns revealed by precipitin studies were initially thought to indicate host preferences (Andersen et al., 1961; Downe, 1960; Tempelis, 1970). Edman et al. (1974) pointed out that attraction should

not be equated with feeding success and more detailed knowledge of the hosts' ecology and behavior is essential for accurate interpretation of feeding patterns. The importance of hosts' defensive behaviors on mosquito feeding success has been repeatedly demonstrated (Edman et al., 1971, 1972, Webber and Edman, 1972). For this and other reasons the concept of host preference is being replaced by one of host availability and acceptability (Wright and DeFoliart, 1970; Edman, 1971).

Still, the findings of field and laboratory studies suggested the existence of some innate preference at least at the attraction level. Bait trap studies indicated preferences for classes of vertebrates (Downe, 1960; Andersen et al., 1961; Tempelis, 1970) but specific preferences were not found (Dow et al., 1957). Others have demonstrated, in the laboratory, differential attraction to various hosts (Gillies, 1964, McIver, 1968, Khan et al., 1970; Gouck, 1972).

Many investigators have shown the importance of host size and density in influencing host preference (Dow et al., 1957; Downe, 1960; McIver, 1968; Edman and Webber, 1975).

Host Age and Mosquito Attraction

Few researchers have actually investigated the

importance of host age in mosquito attraction. The predominant studies concern differential feeding rates to adult and young humans by anophelines (Clyde and Shute, 1958; Muirhead-Thomson, 1951; Boreham et al., 1978). Maibach et al. (1966) demonstrated differential attraction to various human age groups using their probing time 50 technique. More significantly, McIver (1968) found equal weights of older chicks more attractive than young chicks to Aedes aegypti(L.) and Edman et al. (1974) found baby opossums surprisingly unattractive to Culex nigripalpus Theobald. Blackmore and Dow (1958), investigating feeding success on young and adult birds, concluded that the natural close proximity of adult and nestling altricial birds negates any differences in attraction.

Mosquito Fecundity

Early work on mosquito fecundity has been reviewed in Christophers (1960) and Clements (1963). Greenberg (1951), studying the nutritive value of washed sheep erythrocytes, found an increase in fecundity when supplementations of protein or the amino acid isoleucine were made. Later it was demonstrated that eight amino acids were critical to egg development. Isoleucine was the most important of these (Lea et al., 1956; 1958;

Dimond, 1956).

Other factors have been shown to influence mosquito fecundity. Mosquito age was found to have a negative correlation with fecundity (Woke et al., 1956; Stahler et al., 1971; Jalil, 1974) and most researchers have reported good correlation between blood meal size and fecundity (Barlow, 1955; Woke et al., 1956; Colless and Chellapah, 1960; Shelton, 1972; Jalil, 1974). Boorman (1960) pointed out the problems with the gravimetric techniques used to determine blood meal size. A more accurate hemoglobinometric technique has been developed (Briegel et al., 1978).

Numerous investigators have reported greater fecundity on blood meals from avian and reptilian hosts (Woke, 1937; Halcrow, 1951; Colless and Chellapah, 1960; Bennett, 1970; Stahler and Seeley, 1971; Shelton, 1972; Jalil, 1974). Recently, Chang and Judson (1977, 1979) demonstrated that these differences were due to the varying levels of isoleucine found in hosts' blood.

Although Woke et al. (1956) found no host-associated factors important in determining fecundity, host age has been shown to influence fecundity in other hematophagous arthropods (Buxton, 1948; DeMeillon and Hardy, 1951; Rothschild and Ford, 1972). Also Sutherland and Ewen (1974) reported a decrease in fecundity from mosquito-sensitized mammals.

C H A P T E R II

THE EFFECT OF HOST AGE ON MOSQUITO ATTRACTION AND FEEDING

Abstract

Aedes aegypti(L.) attraction to four species (laboratory rat, domestic rabbit, Eastern cottontail, *Sylvilagus floridanus* (Allen), opossum *Didelphis marsupialis* L.) of adult and maturing young hosts was evaluated, using convective air flow olfactometers. Young and adult hosts were compared in both convective air flow olfactometers and a flight-tunnel olfactometer. Laboratory observations of single mosquito responses to these hosts were also made.

Mosquitoes were attracted to all ages of three of the four host species tested. Opossums in the marsupium were not attractive when removed and tested in convective air flow olfactometers, but no evidence for repellency was found. Levels of attraction increased with body development and critical weights were found for each species after which attraction to the adult and young host was comparable. Litters were as attractive as, or more attractive than the adult.

Mosquitoes readily engorged on all young hosts. Young animals displayed fewer and less effective defensive behaviors than the corresponding adults.

Introduction

Detailed knowledge of host-vector interaction is incomplete (Reeves 1971) and the epidemiological importance of immature, non-immune, hosts is well documented (Issel 1974, Gauld *et al*. 1974). Although many researchers have studied mosquito attraction to various host species (McIver 1968, Khan *et al*. 1970, Wright and DeFoliart 1970, Edman 1979), few have investigated the effect of host age on mosquito attraction (Muirhead-Thompson 1951, Maibach *et al*. 1966, McIver 1968). Edman *et al*. (1974) reported surprisingly little feeding by *Culex nigripalpus* Theobald on baby opossums. As an explanation they suggested the possibility of either missing attractive factors or a natural repellency in these young hosts.

The study was conducted to determine whether or not natural repellency exists in certain young mammals and to observe mosquito attraction during the development of young hosts. Direct observations of mosquito feedings on young hosts and defensive behaviors of young hosts were also made.

Materials and Methods

Mosquitoes. *Aedes aegypti* (L.) Georgia strain, which have

been continuously maintained in our laboratory on human blood, were used in most experiments. Larvae were reared in enamel pans at a density of 250 larvae per liter of water per pan. A larval diet of 1:1 Brewer's yeast and lactalbumin was used. Adults were maintained on 2% sucrose and were sugar-starved, but not water-starved, 12 hours before testing. The rearing room was maintained at 27°C, 75-80% RH, with a 14:10 h light:dark cycle (light cycle illumination 1076 lux).

Newly colonized Aedes triseriatus (Say) from Vero Beach were used in one experiment and were reared similarly to the Ae. aegypti. Only mosquitoes 6 to 8 days old and responding to a human hand were used.

Host. The following host animals were used in this study: laborabory rat, domestic rabbit, Eastern cottontail rabbit (Sylvilagus floridanus (Allen)) and opossum (Didelphis marsupialis L.). Domestic animals were bred in our laboratory and fed on standard commercial diets (Purina Laboratory Chow, Purina Rabbit Chow). Suckling cottontails were collected from nests and hand-reared on a milk/vitamin mixture. Adult cottontails were fed rabbit chow and fresh greens. The opossum was live-trapped carrying young and maintained on a diet on dry dog food (Purina Dog Chow), live rodents and fruit. The age of cottontail and opossum young were estimated by criteria found in the literature

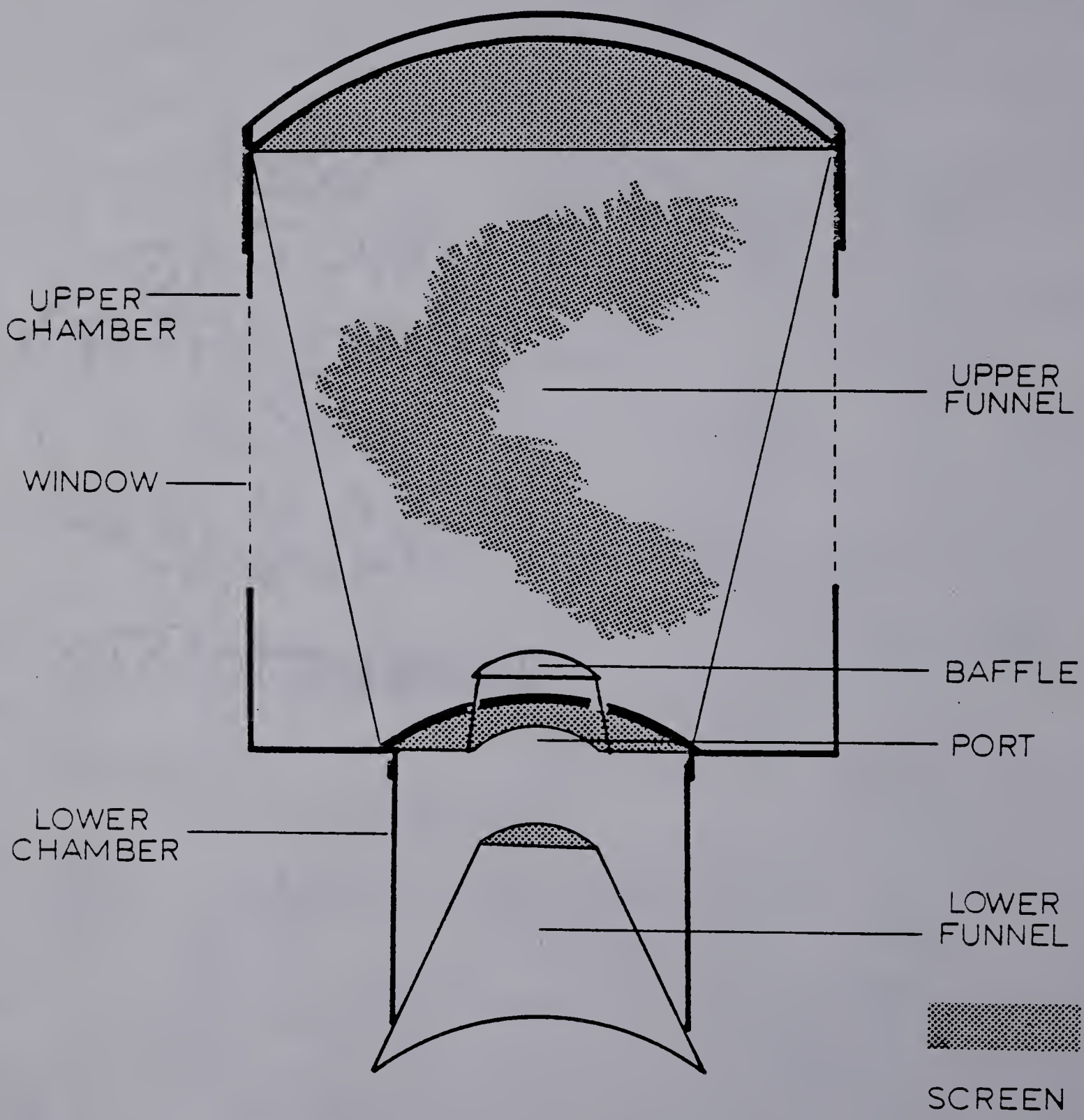
(Beule and Studholme 1942, Reynolds 1952). All animals were cage adapted before testing.

Olfactometer tests. Two olfactometer types were utilized. Convective air flow olfactometers (Figures 1,2), designed by Feinsold and Spielman (1979) and modified with solid baffles, were used to assess mosquito attraction to adult and young hosts. This olfactometer can be used to quickly distinguish between close-range attraction, no response, and repulsion without the behavioral complications encountered in forced-air olfactometers. An adult and young of each species were compared throughout the development of the young animal. Mosquito response to the adult served as a control and the test series was terminated when comparable responses to adult and young hosts were observed. In other tests adult hosts were compared with groups of young hosts. Tests on laboratory rats were conducted with Aedes triseriatus as well as Aedes aegypti.

During testing, the olfactometer was mounted on a plywood host box measuring 45 cm x 37 cm x 30 cm (Figure 3). The box was fitted with a removable front (A), host cage (B), and a metal pyramidal funnel (C) which functioned to direct host emanations into the lower chamber of the olfactometer. For simultaneous testing of adult and young hosts two boxes and olfactometers were used. These were arranged one m apart on a countertop in the open laboratory. Environmental

Figure 1. Diagram of the convective air flow olfactometer.

CONVECTIVE AIR FLOW OLFACTOMETER
Feinsod and Spielman 1979

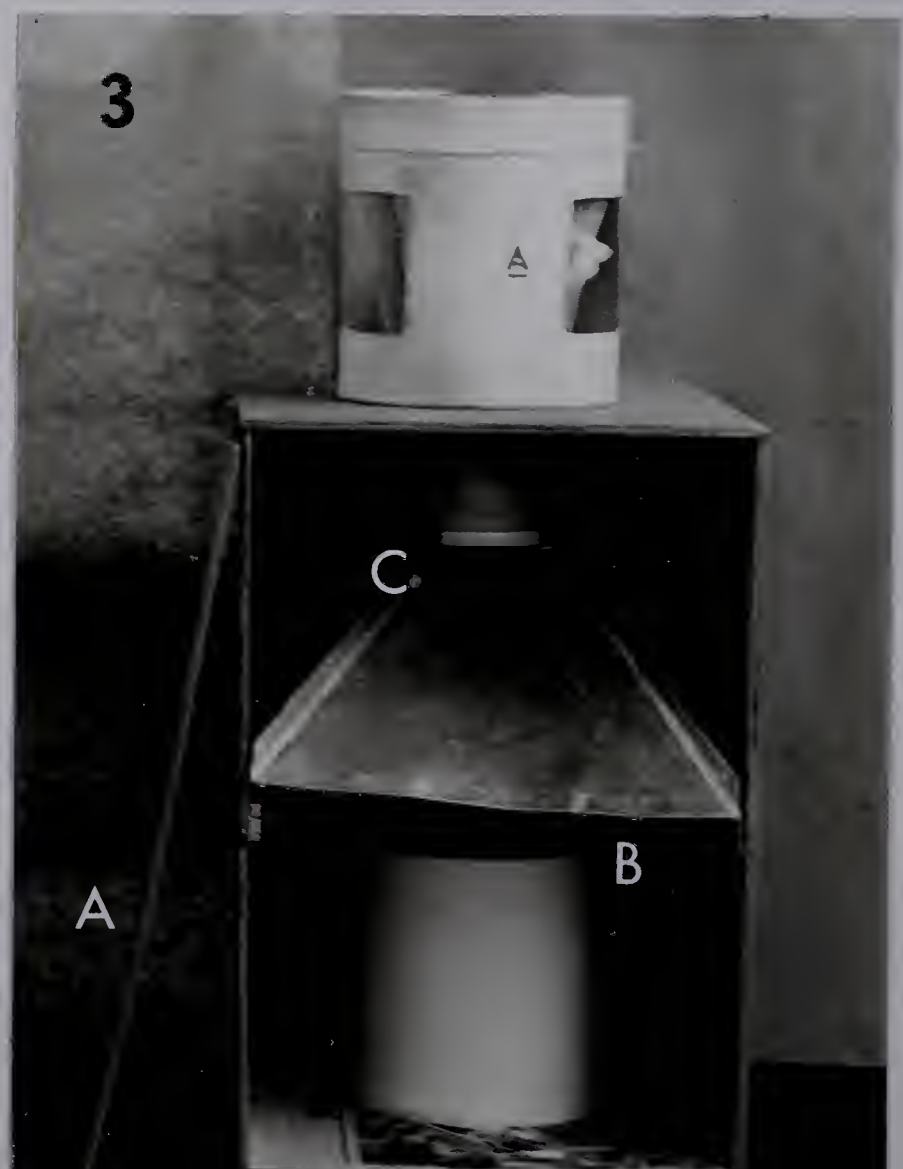


CUT-AWAY VIEW

Figure 2. Intact and dismantled convective air flow olfactometers.

Figure 3. Convective air flow olfactometer in position on the host box. (A) Removable front, (B) host cage, and (C) pyramidal funnel.


2
A


3
A
C
B
A

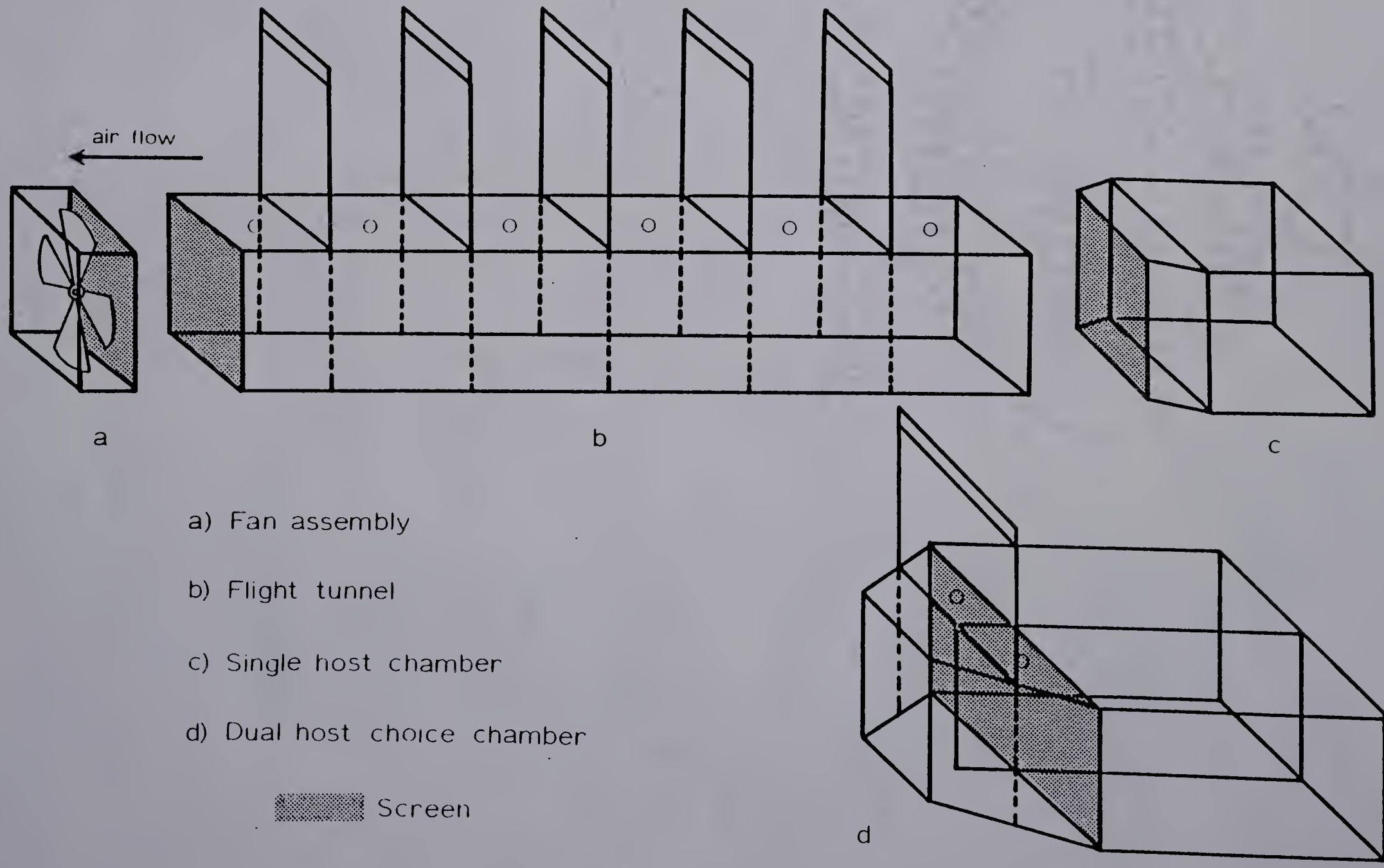
factors in the room could not be controlled but experiments were performed at a room temperature of 20° ± 2°C. Room temperature and air temperature 10 cm above each host were recorded. Approximately twenty mosquitoes were then gently aspirated into the upper chamber of each olfactometer. After an acclimation period of 5 min. the olfactometers were mounted on the host boxes for a test period of 5 min. The number of mosquitoes in the lower chamber and the total number of mosquitoes were recorded. The percentage of mosquitoes in the lower chamber served as the index of attraction. Twelve replications were carried out each test day and host positions were alternated between replications.

To compare host selection when an adult and young host are in close proximity, a Plexiglas^{R} flight tunnel olfactometer, based on a design by Mayer and James (1969), was used. The flight-tunnel olfactometer (Figure 4) was modified with a choice chamber (D) which allowed for simultaneous testing of two hosts in close proximity. The tunnel (B) was 1.76 long, 31 cm high, and 25 cm wide. It is divided into six compartments by 5 sliding doors. Air from outside the building was drawn through the olfactometer by a fan (A) and the host choice chamber was attached to the upwind end of the tunnel. This chamber consisted of two parallel host compartments and a tapered mosquito-trapping section.

Figure 4. Diagram of the Plexiglas^{R} flight tunnel olfactometer.

FLIGHT-TUNNEL OLFACTOMETER

Mayer and James 1969



a) Fan assembly

b) Flight tunnel

c) Single host chamber

d) Dual host choice chamber

Screen

The host compartments measured 31 cm x 31 cm x 54 cm and was separated from the rest of the chamber by screening. A sliding door 14 cm downwind from the host compartments divided the mosquito trapping section and trapped mosquitoes in the 2 adjacent compartments. Mosquito were removed through ports in each compartments.

Before experiments were performed, the olfactometer was flushed for fifteen min by drawing clean air through it. Caged hosts were then placed in the host compartments and the air speed set at a rate of 1800 cm/min. Temperature of incoming and emerging air from both host compartments were recorded. The sliding doors were closed and approximately 50 mosquitoes were aspirated into the downwind end compartment. After a 10 min acclimation period, the doors were opened for a 10 min test period. Mosquitoes trapped at the port to each host compartment were counted. The percentage of mosquitoes trapped at each port served as the index of attraction.

With this olfactometer we compared the mosquito response to an adult laboratory rat with response to both a single young and an entire litter. Six replications were made for each test and the olfactometer was flushed and host positions were alternated between each replication.

Host selection experiments were also performed in still air. For these tests three adjacent compartments of

the flight tunnel were used. Young hosts were placed in half-pint paper containers and positioned in the end compartments. The air temperature of each compartment was recorded and approximately 50 mosquitoes were aspirated into the center compartment. After an acclimation period of 15 min the doors to the center compartment were opened for a test period of 15 min. Again, the percentage of mosquitoes in each host compartment served as the index of attraction.

The following host situations were investigated: young opossum versus control (empty container), young opossum versus young rat of comparable weight, and the same opossum and rat one week later. For each test, 10 replications were made, and host positions were alternated between replications.

Single mosquito/host observations. Observations were made in a Plexiglas^{R} box measuring 30 cm x 30 cm x 45 cm, with a screened top and an open bottom. Small hosts were placed directly on a counter top while larger hosts were held in a plastic box similar in size to the observation box. Temperatures within the observation box and 4 cm above each host were recorded. One female mosquito was then aspirated into the observation box. After an acclimation period of 5 min the observation box was placed over the host and the following observations were recorded: time from host

introduction to flight activity (TA), time from flight initiation to landing on host (LT), feeding, feeding location on host, feeding time from first observable abdominal distention to repletion (FT), and host defensive behavior. Observations were made on both adult and young hosts.

In all experiments hosts were weighed just before testing and plastic gloves were worn throughout all handling of host animals and experimental apparatus.

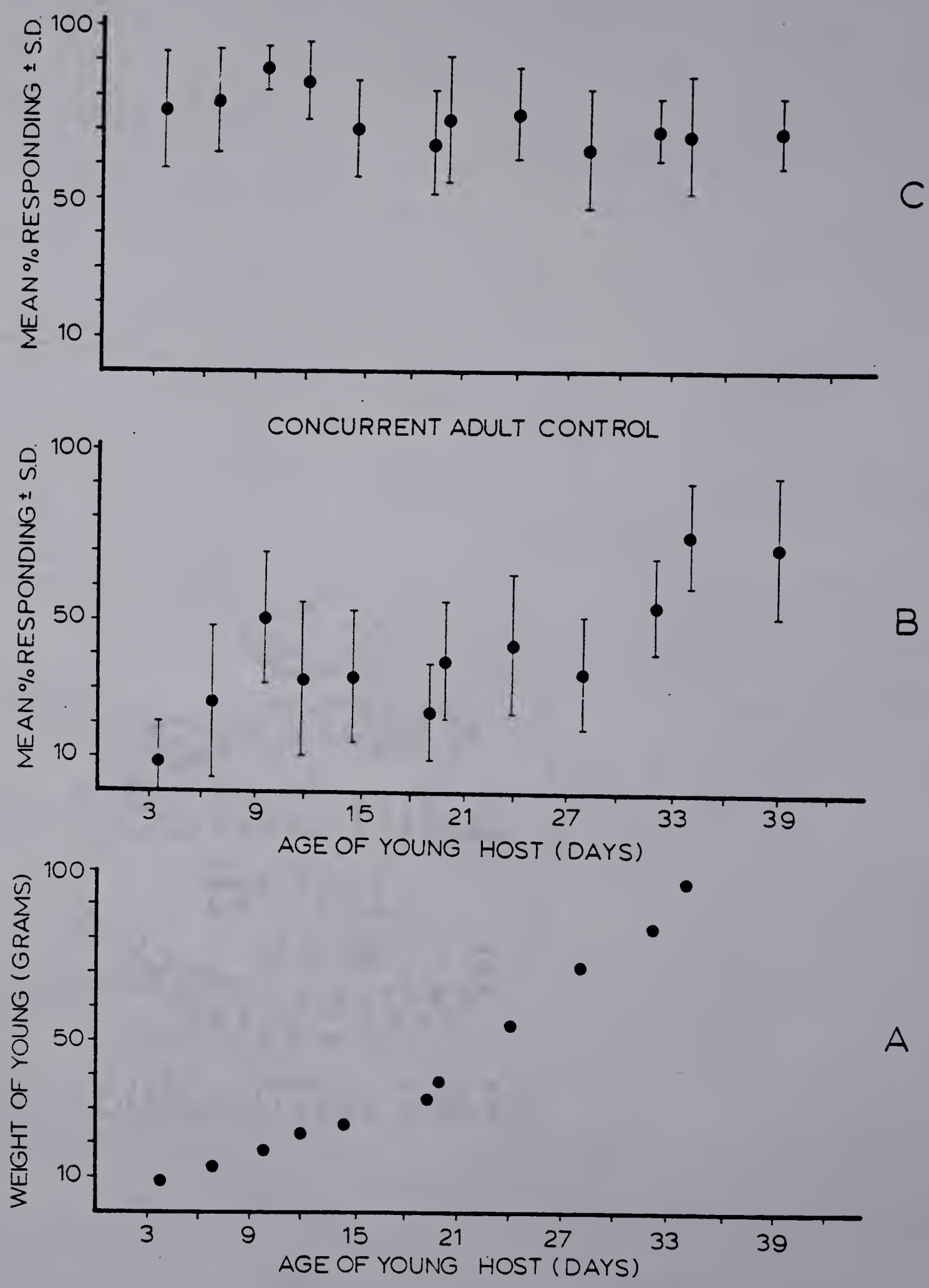
Statistical Analysis. All percentage data were normalized by arcsine transformation. Regression analysis of host age and mosquito response was performed and the product-moment correlation coefficient for the host weight and mosquito response relationship was calculated for each host. Where appropriate, the Student t-test or analysis of variance was used for significance testing.

Results and Discussion

Single host attraction.

Laboratory rat. The relationship between mosquito attraction and the development of the young rat is shown in Figure 5B. Regression analysis of these data (normalized by arcsine transformation) gave a curve with a slope of 0.778, which is significantly different from zero at the 1% level. A similar analysis of data for attraction to the

Figure 5. (A) Weight of and (B) mosquito (Ae. aegypti) response to developing young laboratory rats and (C) mosquito response to adult laboratory rats (control).


LABORATORY RAT
MEAN % RESPONDING ± S.D.
100
50
10
C
CONCURRENT ADULT CONTROL
MEAN % RESPONDING ± S.D.
100
50
10
3
9
15
21
27
33
39
AGE OF YOUNG HOST (DAYS)
B
WEIGHT OF YOUNG (GRAMS)
100
50
10
3
9
15
21
27
33
39
AGE OF YOUNG HOST (DAYS)
A

adult host (Figure 5C) gave a curve with slope of zero ($P<0.01$). This positive relationship of mosquito attraction to host age disappeared as the young host reached 34 days old. The physical parameters possibly associated with this phenomenon are numerous and the quantification of most of these was beyond the scope of this study.

However, we did record the weight of the developing young rat and found a highly significant correlation ($r=0.787$, $P < 0.001$) between this factor and mosquito attraction. The young rat's weight at 34 days old (96 gm) was approximately one third of the adult weight and may represent a critical weight at which attraction to adult and young hosts is equalized. Edman and Webber (1975) pointed out the importance of host size — either as weight or surface area — in mosquito attraction. Besides the visual factors, host size influences levels of metabolic products (heat, moisture, CO_2, chemical emanations) known to be attractive. Also convective currents, suggested by some investigators to be a primary factor in host attractancy, are obviously affected by host size (Wright and Kellogg 1964).

Observations of single mosquito attraction to adult and young rats are summarized in Table 1. Although the percentages of mosquitoes stimulated into flight were similar, the reaction time to the young rat was approximately twenty seconds slower. Thirty-six percent fewer mosquitoes

TABLE 1 Observations on the responses of individual mosquitoes to adult and young laboratory rats.

Host	N	ΔTemp. °C[b] $\bar{X} \pm$ SD	% Active T.A.(sec) $\bar{X} \pm$ SD	% Landing L.T.(sec) $\bar{X} \pm$ SD	% Feeding F.T.(sec) $\bar{X} \pm$ SD	Feeding Location (%)	Defensive Behavior
Young Rat (6 Days)	23	0.59 ± 0.47	87.0 58.0 ± 42.0	56.5 100.5 ± 82.2	52.2(83.3)[a] 208.0 ± 60.0	27.2 Head 36.4 Rump 36.4 Back	Food scratch
Adult Rat	15	1.93 ± 0.26	93.3 36.6 ± 35.1	93.3 38.1 ± 59.2	60.0(66.7) 177.5 ± 66.0	11.1 Eyelid 22.2 Foot 66.7 Tail	Body shake Foot scratch

[a] Numbers in parentheses represent percentage of feedings actually completed.

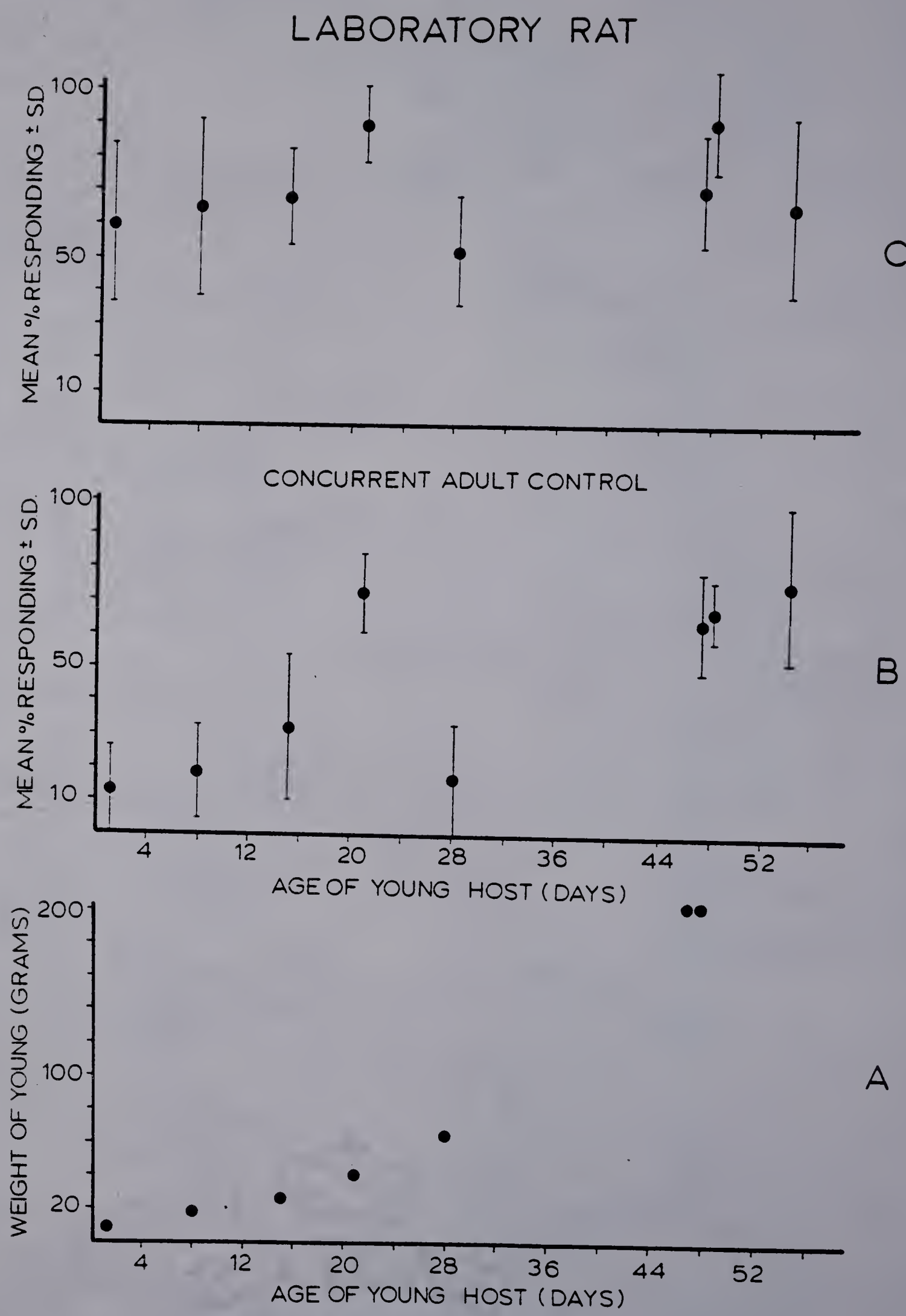
[b] Host ambient temperature minus room ambient temperature.

landed on the young rat than on the adult, and the mean landing time with the young rat was nearly triple that with the adult. These findings indicate that young rats stimulate mosquitoes to fly as readily as adult rats, but the young seem somewhat deficient in cues guiding the mosquito to land.

Temperature gradients have been suggested as guidance in close-range attraction (Hocking 1971). We observed temperature differentials between young and adult hosts but these differences could not account for the pattern of mosquito attraction observed during rat development. Other size-associated factors already mentioned may have been responsible for the differences but these were not investigated. Since Khan (1957) found differential attraction to hosts fed on various diets, we were interested in the possible effect weaning might have on mosquito attraction. No discernible alteration in the response curve was found at weaning (approx. 21 days old). Sexual hormones (Roessler and Brown 1964) were apparently not influencing attraction, since sexual maturity in the rat occurs well after (50-60 days) the 'attractiveness maturity' (34 days) observed.

Results of a similar developmental study of attraction to the laboratory rat by Aedes triseriatus are shown in Figure 6. Again, a positive relationship of mosquito

Figure 6. (A) Weight of and (B) mosquito (Ae. triseriatus) response to developing young laboratory rats and (C) mosquito response to adult laboratory rats (control).


LABORATORY RAT
MEAN % RESPONDING ± SD
100
50
10
C
CONCURRENT ADULT CONTROL
MEAN % RESPONDING ± SD.
100
50
10
4
12
20
28
36
44
52
B
AGE OF YOUNG HOST (DAYS)
WEIGHT OF YOUNG (GRAMS)
200
100
20
4
12
20
28
36
44
52
A
AGE OF YOUNG HOST (DAYS)

attraction to host age ($b=0.665$, $P < 0.05$) and significant correlation ($r=0.741$, $P < 0.05$) between host weight and mosquito attraction were found for the young rat. However, in this case the mosquito response to young and adult rats was not equalized until the young host was 54 days old or 230 grams. The similarity in responses of these two mosquito species should minimize the possibility that observed patterns of attraction to developing hosts were artifacts of continued colonization. Disparity in these results may be due to the smaller sample size or to different species utilization of attractive stimuli (McIver 1968, Omer 1979).

Opossum. As with the young laboratory rat, mosquito attraction to the young opossum was found to have a positive relationship with host age (Figure 7, $b=0.747$, $P< 0.001$). A significant correlation ($r=0.711$, $P < 0.01$) between host weight and mosquito attraction was also found, and both these relationships were lost when the young host became 140 days old or 770 grams. Unlike the rat, very young opossums were not attractive when tested in the convective air flow olfactometer. This lack of attractancy lasted until the young opossums left the marsupium about the 76th day.

In mosquito dispersal experiments (Table 2), in which visual stimuli were controlled, mosquitoes were attracted to a 69-day-old opossum. When given a choice between a young opossum,76 days old, and a young rat of comparable weight,

Figure 7. (A) Weight of and (B) mosquito (Ae. aegypti) response to developing young opossums and (c) mosquito response to adult opossum (control).

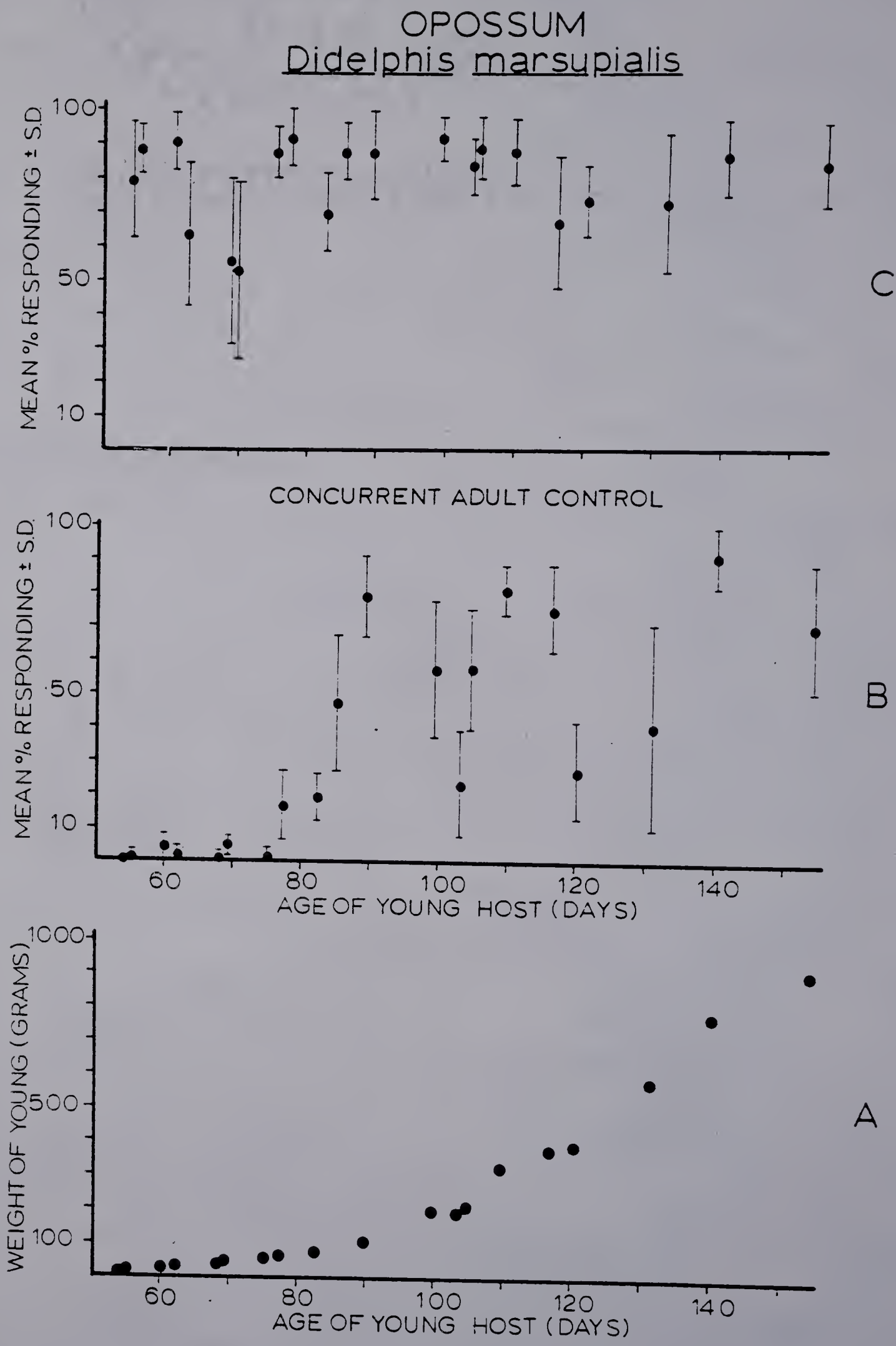

OPOSSUM
Didelphis marsupialis
MEAN % RESPONDING ± S.D.
100
50
10
C
CONCURRENT ADULT CONTROL
MEAN % RESPONDING ± S.D.
100
50
10
60
80
100
120
140
AGE OF YOUNG HOST (DAYS)
B
WEIGHT OF YOUNG (GRAMS)
1000
500
100
60
80
100
120
140
AGE OF YOUNG HOST (DAYS)
A

TABLE 2 Results of host selection by mosquito dispersal experiments. Opossum versus laboratory rat.

Test Situation	N	Opossum Weight (Grams)	Opossum Age (Days)	Opossum % Mosq. Response $\bar{X} \pm SD$	Laboratory Rat Weight (Grams)	Laboratory Rat Age (Days)	Laboratory Rat % Mosq. Response $\bar{X} \pm SD$	Control % Mosq. Response $\bar{X} \pm SD$
Opossum vs C[a]	10	24	69	45.1 ± 14.9*	-	-	————	30.4 ± 11.1
Opossum vs Rat	10	38	76	8.9 ± 6.9	43	20	73.5 ± 12.6**	————
Opossum vs Rat	10	111	95	41.2 ± 15.5 NS	118	39	47.9 ± 14.3	————

[a] Control.

* Significant at the 0.05 level.

** Significant at the 0.01 level.

NS Not significant.

the mosquitoes showed a significant ($P < 0.01$) preference for the young rat. No preference for either host was found when this experiment was repeated 19 days later. Observations of mosquito attraction to adult and 87-day-old opossums suggested little difference in the attractancy of these hosts (Table 3).

Reynolds (1952) demonstrated that temperature regulation in the young opossum begins around 70-80 days of age. It is perhaps this inability of the young opossum to maintain its body temperature that accounts for such low-level mosquito attraction to opossums younger than this age. No evidence was found to suggest a natural repellency in the young opossum (Edman et al. 1974). Beyond this period of low attractancy, differences in attraction to developing opossums are probably due to size-associated attractive stimuli. Again the events of weaning (approx. 97 days) and sexual maturity are apparently of little or no importance.

Domestic rabbit. No apparent relationship exists for mosquito attraction and the development of young domestic rabbits (Fig. 8). Both mosquito attraction curves have a slope of zero and no significant correlation was found between host weight and mosquito attraction. Young rabbits elicited a lower level of mosquito attraction than did adults, and this response was constant from birth

TABLE 3 Observations on the responses of individual mosquito to adult and young opossums

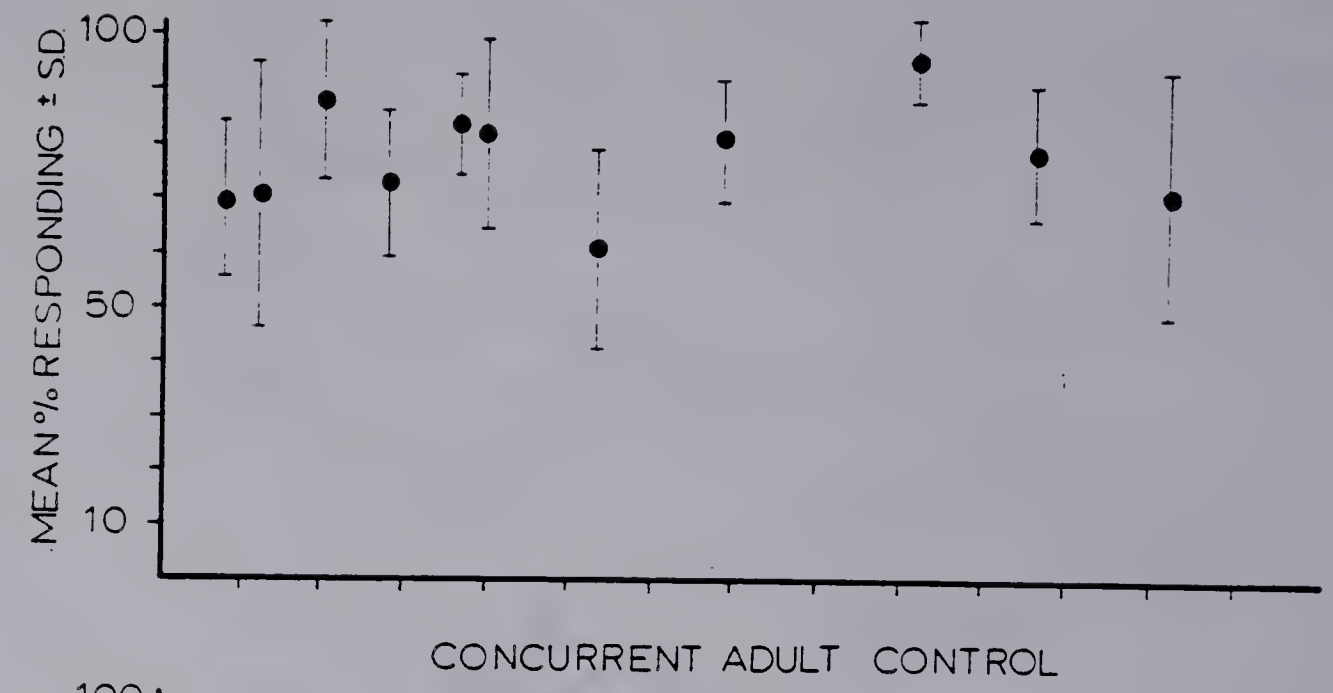
Host	N	ΔTemp.°C[b] $\bar{X} \pm$ SD	% Active T.A.(sec) $\bar{X} \pm$ SD	% Landing L.T.(sec) $\bar{X} \pm$ SD	% Feeding F.T.(sec) $\bar{X} \pm$ SD	Feeding Location %	Defensive Behavior
Young Opossum (87 Days)	18	0.36 ± 0.33	94.4 60.1 ± 56.1	88.9 48.9 ± 46.7	72.2(53.9)[a] 153.6 ± 70.8	23.0 Head 38.5 Leg 38.5 Back	Body shake Foot scratch
Adult Opossum	21	1.95 ± 1.07	100 19.9 ± 16.8	100 39.7 ± 22.0	95.2(40.0) 170.6 ± 63.6	35.0 Snout 35.0 Ear 15.0 Tail 15.0 Foot	Ear flip Foot scratch

[a] Numbers in parentheses represent percentage of feedings actually completed.

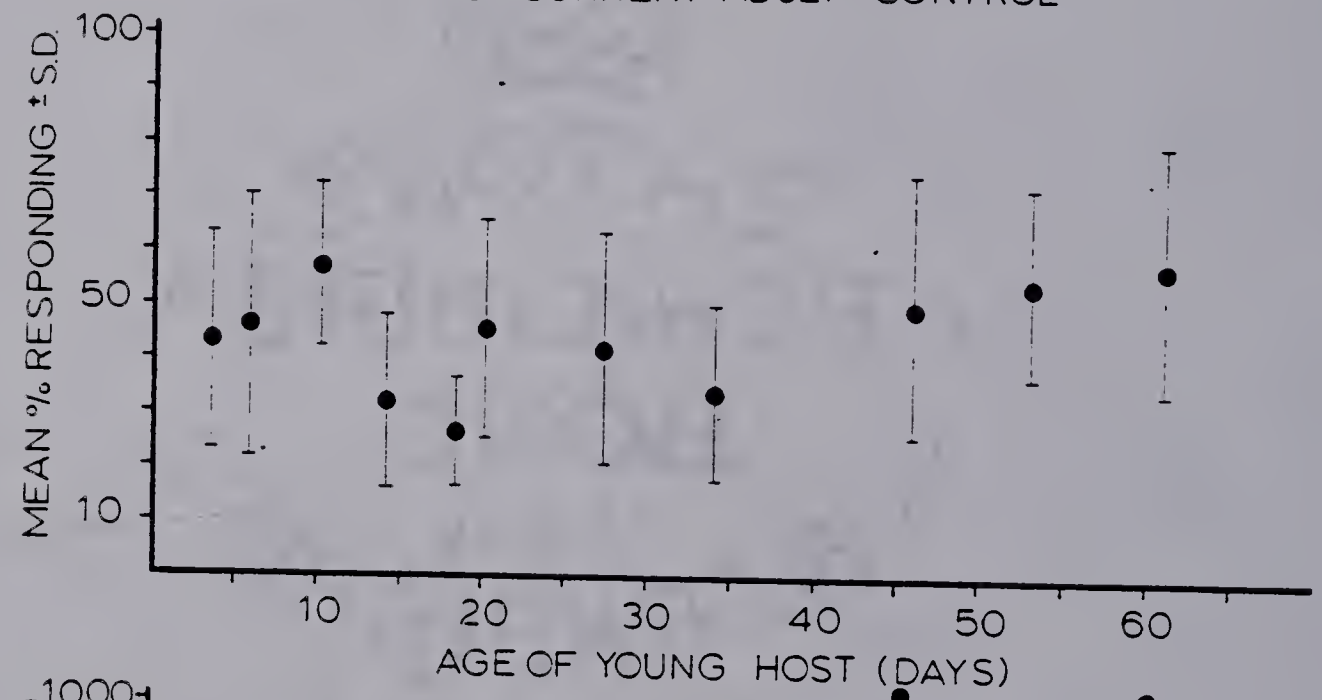
[b] Host ambient temperature minus room ambient temperature.

Figure 8. (A) Weight of and (B) mosquito (Ae. aegypti) response to developing young domestic rabbits and (C) mosquito response to adult domestic rabbit (control).

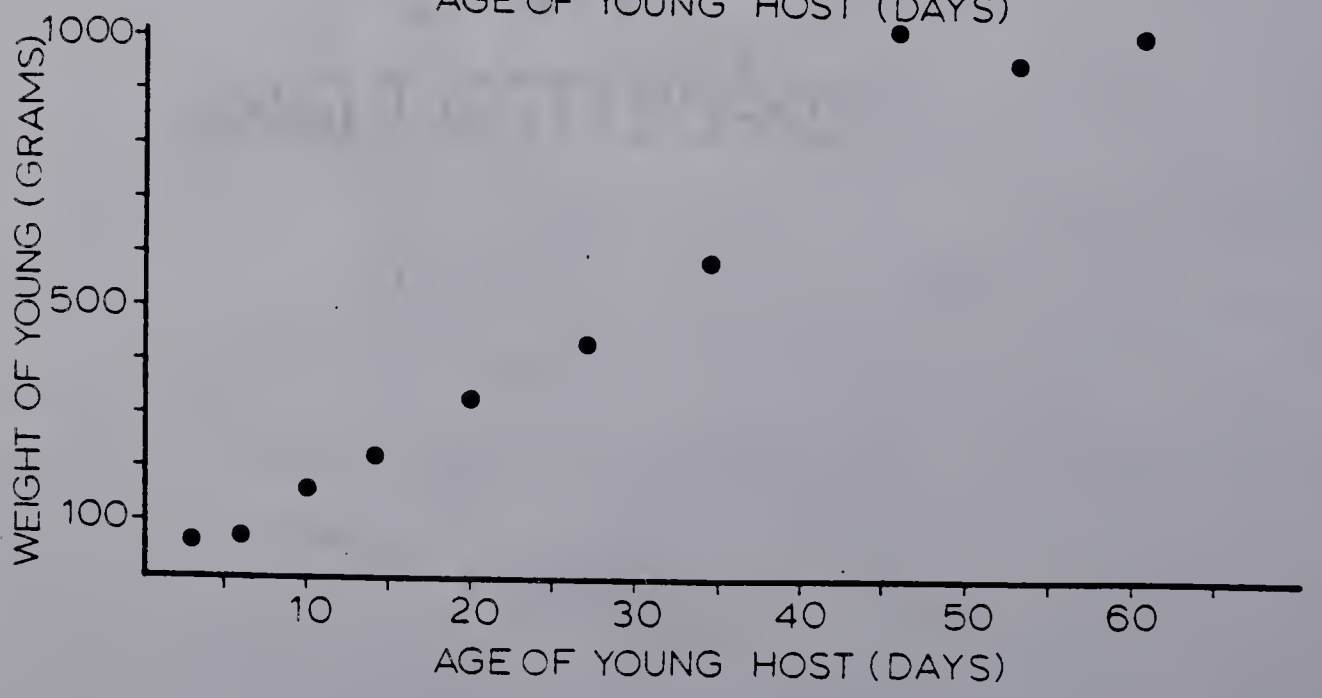
DOMESTIC RABBIT



C



B



A

until 60 days of age. At this point the young rabbits were as attractive as the adult and weighed about one kilogram – ca. one-fourth the adult weight. Neither weaning (approx. 42 days) nor sexual maturity (9-12 months) could be associated with this change in mosquito response.

Although olfactometer studies showed a consistent lower level of attraction to the young rabbit, a newborn rabbit was demonstrated to be as attractive as an adult in single mosquito observations (Table 4). The relatively large size of the newborn rabbit (42 grams), and the relatively short distance (within 60 cm) being studied, may account for the observed equivalence in attractancy. No temperature differential was observed between the two hosts (Table 4). The fifty-three percent fewer landings and the longer reaction times of mosquitoes responding to the adult rabbit was probably due to the large size of the adult and the relatively small space of the observation box. In this situation, confinement of the host's convective currents could reduce gradients of attractive stimuli important in close-range host location.

Cottontail rabbit. A positive relationship for mosquito attraction and host age ($b=0.322$, $P < 0.01$) and a significant correlation ($r=0.681$, $P < 0.05$) between host weight and mosquito attraction were found in the development of young cottontails (Figure 9). Cottontail

TABLE 4 Observations on the responses of individual mosquito to adult and newborn domestic rabbits.

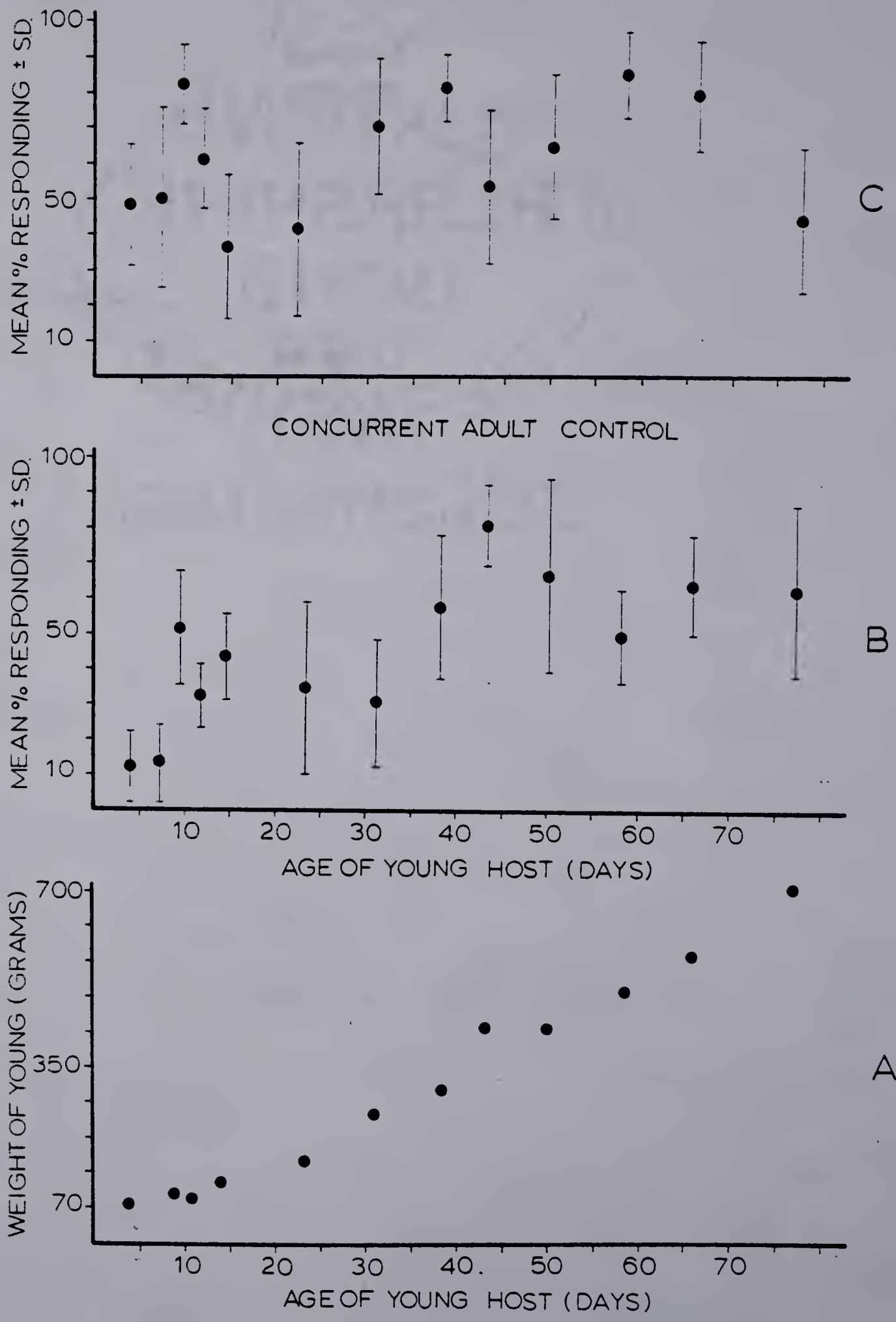
Host		Δ Temp. °C[b] $\bar{X} \pm$ SD	% Active T.A. (sec) $\bar{X} \pm$ SD	% Landing L.T. (sec) $\bar{X} \pm$ SD	% Feeding F.T. (sec) $\bar{X} \pm$ SD	Feeding Location %	Defensive Behavior
Newborn Rabbit (1 day)	20	1.50 ± 0.50	100 23.5 ± 45.1	100 18.5 ± 25.7	100(100)[a] —	—	—
Adult Rabbit	15	1.80 ± 0.40	100 70.7 ± 90.0	46.7 32.9 ± 45.5	26.7(0) —	100 Ear	Ear flip

[a] Numbers in parentheses represent percentage of feedings actually completed.

[b] Host ambient temperature minus room ambient temperature.

Figure 9. (A) Weight of and (B) mosquito (Ae. aegypti) response to developing young cottontail rabbits and (C) mosquito response to adult cottontail rabbit (control).

EASTERN COTTONTAIL
Sylvilagus floridanus



young grow quickly and are weaned at about two weeks of age (Beule and Studholme 1942). Attraction to the young, comparable to that of the adult, was found as early as ten days and was consistently found after 66 days of age (570 gms). Interestingly, mosquito attraction to adult cottontails was lower and more erratic than attraction to adults of other host species.

In mosquito observation studies (Table 5) differences in attraction to young and adult cottontails were also observed and a temperature differential was present. As in the case of the domestic rabbit, mosquitoes appeared to have some difficulty locating the adult cottontail.

Attraction to the litter. Mosquito responses (attraction) to groups of young hosts and to the adult are summarized in Table 6. All young hosts were tested together at an age at which they would naturally be found together (prior to weaning) and before the critical age at which attraction to young and adult hosts became comparable. With the exception of the opossum, all groups of young were shown to be as attractive as the adults. For these hosts the combined weight of the young was well above the weight at which attraction to a single young and adult became comparable. Also, the air temperatures above the young hosts were greater than those above the adult host.

TABLE 5 Observations on the responses of individual mosquitoes to adult and young eastern cottontail rabbits.

Host		ΔTemp. °C[b] $\bar{X} \pm$ SD	% Active T.A.(sec) $\bar{X} \pm$ SD	% Landing L.T.(sec) $\bar{X} \pm$ SD	% Feeding F.T.(sec) $\bar{X} \pm$ SD	Feeding Location %		Defensive Behavior
Young Cottontail (40 days)	18	0.64 ± 0.44	94.4 64.9 ± 77.3	66.7 31.7 ± 32.2	16.6(100)[a] 415.0 ± 8.7	100	Eyelid	Blinking
Adult Cottontail	15	2.07 ± 0.26	100 38.3 ± 25.7	80.0 149.4 ± 86.1	53.3(100) 236.3 ± 124.4	12.5 25.0 25.0 37.5	Foot Eyelid Nostril Ear	Blinking

[a] Numbers in parentheses represent percentage of feedings actually completed.

[b] Host ambient temperature minus room ambient temperature.

TABLE 6 Mosquito response to adult hosts and their litters. Convective air flow olfactometer.

Host	N	Age of Young (Days)	Weight (Grams)	ΔTemp. °C[b] $\bar{X} \pm SD$	Percent Mosquito Response $\bar{X} \pm SD$	
Adult Rat	12	-	323	2.3 ± 0.49	87.1 ± 5.8	
Young Rats(10)[a]	12	6	143	4.2 ± 0.72	83.3 ± 11.4	NS
Adult Opossum	10	-	3500	2.2 ± 0.42	64.46 ± 6.8***	
Young Opossums(5)	10	92	600	1.8 ± 0.39	43.77 ± 10.6	
Adult Rabbit	12	-	4550	2.8 ± 0.39	78.5 ± 8.2	
Young Rabbits(5)	12	38	3480	4.1 ± 0.47	76.0 ± 19.7	NS
Adult Cottontail	12	-	800	2.5 ± 0.43	33.4 ± 21.0	
Young Cottontails(3)	12	17	383	3.2 ± 0.39	60.4 ± 13.8**	

a Figures in parentheses represent the number of young in litter.
b Host ambient temperature minus room ambient temperature.
** Significant at the 0.01 level.
*** Significant at the 0.001 level.
NS Not significant.

In the case of the opossum, the weight of the litter was below the critical weight for comparable attraction, and higher air temperatures were found above the adult than the young. Results of host choice tests, made in our flight-tunnel olfactometer (Table 7), showed mosquitoes significantly ($P < 0.05$) preferred a litter of young rats over an adult rat tested in close proximity. The findings of tests on attraction to host litters further suggest that differences in mosquito attraction to young and adult hosts are based on host size-associated quantities of attractive stimuli and not on the stimuli themselves.

Mosquito feeding and host defensive behavior. Observations of mosquito feedings on young and adult hosts are given in Tables 1,3,4,5. For all the hosts tested except the domestic rabbit, more mosquitoes attempted to feed on the adult host than on the young. Since mosquitoes were observed to feed readily on all of the young hosts, this pattern was thought to be a reflection of the number of mosquitoes landing on the host. A restriction of suitable feeding sites, as well as a low landing rate, may have influenced the low feeding rate observed for the adult domestic rabbit.

Mosquitoes fed on all body regions of the naked young hosts, but were restricted by dense fur to a few suitable sites on the adult host. Feeding on the adult rat occurred

TABLE 7 Results of host choice tests in flight-tunnel olfactometer. Adult versus young laboratory rats.

Test Situation	N	Weight of Young (Grams)	Age of Young (Days)	Percent Mosquito Response	
				Young X ± SD	Young X ± SD
Adult vs. Young	6	16	9	50.0 ± 16.8***	16.9 ± 7.1
Adult vs. Litter (12)[a]	6	190	8	28.1 ± 14.8	51.4 ± 12.3*

[a] Number of young in litter.

* Significant at the 0.05 level.

*** Significant at the 0.001 level.

on the tail, feet and eyelids. Common feeding sites on the opossum were the fleshy ears and short-haired snout. Mosquito feeding on the domestic rabbit was restricted to the ears, but on the cottontail it also included the feet, eyelids and nose.

Finally, host defensive behavior varied both in form and effectiveness with the species and age of the host. Newborn and young rats were observed to scratch at feeding mosquitoes, but this behavior did not prove to be effective since 83 percent of the feedings were completed. Young opossums shook their bodies and scratched to dislodge mosquitoes, and this behavior appeared to be moderately effective. Newborn domestic rabbits, young cottontails and adult cottontails displayed no defensive behaviors, aside from blinking, and as an effect of this, 100% of the mosquito feedings on these hosts were completed. Observed defensive behaviors in other adult hosts were somewhat more elaborate and more effective than those of the young host.

The validity of 'anti-mosquito behavior' in laboratory domesticated animals is questionable, and admittedly, these observations are at best preliminary. Still it is possible to conclude from these observations, as did Kale et al. (1972), that mosquito feeding success is greater on less defensive immature hosts. These findings and

the work of Edman et al. (1974) would further suggest that in nest situations, where adult and young hosts are both available to the mosquito, more feeding is likely to occur on the less defensive young.

Conclusions

No evidence was found for olfactory or gustatory repellency in any of the young hosts tested. Instead, mosquitoes showed measurable attraction to most of the young. Opossums less than 76 days old were the only exception. The level of attraction to the young increased with weight gain and is thought to have been influenced by size-associated quantities of attractive stimuli. At one hundred grams all young hosts were found to attract 50% of the mosquitoes tested, and a critical weight was observed for each young host after which attraction to the young and adult host were comparable. This seems to support the idea that host preference is often related to host size (Downe 1960, McIver 1968, Edman and Webber 1975). However, if this were always the case, then the adult rat should not have been more attractive than the adult cottontail.

Litters of young, with a combined weight greater than the critical weight, were found to be as attractive as, or more attractive than, the adult. Also, because of

their less defensive nature and relative nakedness, young hosts were found more acceptable to feeding mosquitoes than were the adults.

Considering all these observations, it maybe suggested that, in nature, young mammals in a litter are extremely vulnerable to mosquito attack. It seems reasonable to assume that some protection measures have evolved. Vertebrate nest construction may be as much as refection of mosquito ectoparasitism as vertebrate predation and the possibility of maternal defensive behaviors must not be overlooked. From the viewpoint of attraction, it seems clear that young hosts must be included in the population of available hosts, but until mosquito feeding on young is assessed in the field the exact role of young hosts in the ecology of mosquito-borne diseases will remain speculative.

C H A P T E R III

FACTORS AFFECTING DIFFERENTIAL MOSQUITO ATTRACTION TO ADULT AND YOUNG HOSTS

Abstract

Aedes aegypti(L.) attraction to adult laboratory rats was compared with attraction to young rats supplemented with known attractive stimuli (heat, moisture, CO_2, and odors). Attraction was evaluated in both convective air flow olfactometers and a flight-tunnel olfactometer modified with a choice chamber. Individual supplements of heat, moisture or odors did not enhance attraction to the young rats. CO_2 enhanced attraction to young rats in convective air flow olfactometer tests, but only when a .5% CO_2/air mixture was delivered intermittently at 710 ml/min. Results in flight-tunnel olfactometer tests suggest that, with *Ae. aegypti*, CO_2 functions as a stimulator and not as an attractant.

Introduction

Previous studies have attempted to establish a hierarchy of importance for various stimuli in mosquito host seeking behavior. Wright and his co-workers (Kellogg and Wright, 1962; Daykin *et al.*, 1965; Wright, 1975) have set forth a theory considering warmth and moisture the

primary factors influencing attractancy. Many investigators have questioned this concept (Acree et al., 1968; Omer, 1979) and recently Price et al. (1979) concluded that chemical emanations other than carbon dioxide and moisture are the primary attractive factors. Most of these studies have been performed in complicated olfactometers and seldom utilize living hosts. McIver (1968) studied the relative importance of various stimuli in determining laboratory-observed host preferences of Aedes aegypti(L.) and Culex tarsalis Coquillett.

In an earlier study (Chapter II) attractive differences between adult and young mammalian hosts were determined. The objective of this study was to determine what factor(s) was responsible for reduced attraction to young hosts. The stimuli of heat, humidity, carbon dioxide, and odor were investigated using both a convective air flow olfactometer and a wind-tunnel olfactometer.

Materials and Methods

Aedes aegypti(L.) of the Georgia strain were reared as previously described (Chapter II). All adult mosquitoes were 6-8 days old and were sugar-starved but not water-starved for 12 hr before testing.

Convective air flow olfactometers, designed by Feinsod and Spielman (1978), and a Plexiglas^{R} wind-tunnel

olfactometer, based on a design by Mayer and James (1969) were used to assess attraction. The wind-tunnel olfactometer was modified with a choice-chamber which allowed simultaneous testing of two hosts in close proximity. Both olfactometers and test techniques are described in greater detail elsewhere (Chapter II).

Briefly, two convective air flow olfactometers were mounted on two wooden boxes each containing a caged host. Approximately twenty mosquitoes were aspirated into the upper chamber of the olfactometer. After an acclimation period of 10 min the olfactometer was placed above the host for a 5 min test period. Mosquitoes in the upper and lower chambers were then counted and the percentage in the upper chamber served as the index of attraction. Twelve replications were made and hosts were reversed between each test.

In tests with the wind-tunnel olfactometer air was drawn through at a speed of 1800 cm/min. Approximately fifty mosquitoes were aspirated into the release chamber. After an acclimation period of 15 min the doors were opened for a 10 min test period. Mosquitoes at host chambers were counted and the percentage of mosquitoes at each chamber served as the index of attraction. Six replications were made and hosts were reversed between each test.

In all experiments an adult and a young (32 days or less) laboratory rat were tested simultaneously. The adults served as a control for the young supplemented with single stimuli. The stimuli tested and the methods of their introduction were the following.

Heat. This factor was supplied by a 50 ml Erlenmeyer flask, filled with water and heated prior to testing in a water bath. The flask was stoppered, dried, inverted and placed in close proximity to the young rat. When the ambient temperature of both the adult and young host chambers were comparable the test was initiated.

Humidity. Increased humidity in the young host chamber was accomplished by placing a 500 ml beaker filled with distilled water inside the chamber. The water was vigorously aerated with pumped filtered room air.

CO_2. Carbon dioxide was introduced at 3 different rates. Initially, compressed gas was delivered in a constant stream from a CO_2 cylinder at an undetermined rate. Next 120 ml/min was introduced intermittently at 20 second intervals. Finally a mixture of 0.5% CO_2 in filtered air was introduced intermittently at a rate of 710 ml/min.

Odor. Twelve adult laboratory rats were sacrificed by cervical dislocation and immediately rinsed in an

acetone bath. The acetone was then evaporated till 25 ml of solution remained. To deliver the scent into the chamber, pieces of filter paper (3 cm diameter) were saturated with 20 drops of the solution and dried. A similar piece of paper receiving 20 drops of acetone served as the control. After drying, the papers were moistened with 5 drops of distilled water and placed on host water flasks. Flasks and papers were then placed in their appropriate host chambers. The heated skin washings had a distinct rat odor to the human observer.

Heat and CO_2 were tested in both olfactometers, but humidity was only tested in the convective air flow olfactometer and odor only in the wind-tunnel olfactometer. Control experiments using the convective air flow olfactometer were conducted on adult and young rats without supplemental stimuli. Comparisons between these and stimuli-supplemented tests gave an indication of the relative effect the stimuli had on attraction.

All percent data underwent arcsine transformation and were then subjected to Student t-tests for significance (Sokal and Rohlf, 1969).

Results

Mosquito responses to various stimuli presented in the convective air flow olfactometer are summarized in

Tables 8 and 9. In tests where heat or humidity were supplemented to the air streams of young hosts the stimulus was not sufficient to equalize mosquito responses to the adult and young host (Table 8). Carbon dioxide added at a constant rate or pulsed at 120 ml/min appeared to reduce attraction to the young host (Table 9). Mosquito response to the adult and young host was equalized, however, when a mixture of 0.5% CO_2 in filter room air was introduced intermittently at a rate of 710 ml/min.

Table 10 summarizes mosquito response data from wind-tunnel olfactometer choice tests. In these tests no stimulus additions to young hosts were able to equalize responses to the adult and young hosts. Treatments appeared to have no effect on mosquito responses, since no significant differences were found in responses to adult or young host between the control and any of the treatment groups.

Discussion

Numerous investigators (Brown, 1966; Bar-Zeev, 1977) have demonstrated that heat alone or in conjunction with other stimuli is attractive to Ae. aegypti. In the convective air flow olfactometer, host heat also plays a critical role in transportation of host

TABLE 8 Mosquito response to young rat supplemented with heat and humidity. Convective air flow olfactometer. (N = 12).

Treatment	Age of Young (Days)	Mean Percent Response ± SD Adult	Young	Ratio of Response to Hosts Adult/Young
Young (Control)	7	78.0 ± 14.8***	27.3 ± 22.0	2.86
Young & Heat	5	92.0 ± 12.0***	28.1 ± 16.5	3.27
Young (Control)	10	88.2 ± 6.4***	50.8 ± 18.5	1.74
Young & Humidity	11	88.4 ± 7.3***	34.6 ± 13.0	2.55

*** Significant at the 0.001 level.

TABLE 9 Mosquito response to young rat supplemented with various treatment of CO_2. Convective air flow olfactometer. (N=12).

Treatment	Age of Young (Days)	Mean Percent Response + SD Adult	Young	Ratio of Response to Hosts Adult/Young
Young (Control)	8	89.7 ± 8.07***	51.7 ± 22.1	1.74
Young + CO_2[a] (constant rate)	10	62.9 ± 22.4 ***	0.4 ± 1.2	157.25
Young (Control)	7	78.0 ± 14.8 ***	27.3 ± 22.0	2.86
Young + CO_2 (pulsed, 120 ml/min)	6	77.3 ± 10.9 ***	16.7 ± 14.3	4.63
Young (control)	28	74.9 ± 12.4 ***	29.6 ± 12.6	2.53
Young + CO_2 (pulsed, .5% CO_2 710 ml/min)	31	45.7 ± 21.5 NS	38.0 ± 23.0	1.20

[a] Only 10 replications.

*** Significant at the 0.001 level.

NS Not significant.

TABLE 10 Mosquito response to young rat supplemented with heat, CO_2 and odor. Wind tunnel olfactometer, choice test.

Treatment	N	Age of Young (Days)	Mean Percent Response ± SD		Ratio of Response to Hosts
			Adult	Young	Adult/Young
Young (Control)	6	8	49.98 ± 16.84**	16.93 ± 7.14	2.95
Young + Heat	4	8	59.25 ± 5.37**	20.70 ± 7.60	2.86
Young + CO_2[a]	6	13	55.28 ± 6.37**	25.32 ± 6.15	2.18
Young + Odor	6	13	57.22 ± 13.06**	17.07 ± 11.94	3.35

[a] 0.5% CO_2 in air 710 ml/min and pulsed.

** Significant at the 0.01 level.

emanations. It is surprising, then, that the supplementation of heat did not enhance attractancy to the young host.

Not as surprising was the ineffectiveness of increased humidity in enhancing host attractancy. Bar-Zeev (1960) proposed that mosquito attraction to varying degrees of relative humidity in early host-seeking studies was the result of water stress in the test mosquitoes. More recent studies, cautious of this factor, have since demonstrated no attractancy to various humidities when presented as a single stimulus (Bar-Zeev et al., 1977; Price et al., 1979).

Perhaps the most complex element of mosquito host seeking behavior is the role of CO_2. Willis and Roth (1952) found all levels of CO_2 repellent in a small olfactometer, while findings of Kellogg (1970) and Omer (1979) suggest that only oscillating levels of CO_2 are useful cues to the host-seeking mosquito. This may account for the noticeable reduction in response to the young host when supplemented with a constant level of CO_2. Intermittent delivery of CO_2 at a rate as high as 120 ml/min may not have dispersed sufficiently in the convective air flow olfactometer to be perceived by Ae. aegypti in variable concentrations.

The apparent enhancement of host attractancy observed, when a 0.5% CO_2/air mixture was intermittently introduced,

agrees with other findings where enhanced mosquito responses to odors were achieved by adding CO_2 (McIver, 1968; Bar-Zeev et al., 1977; Omer, 1979). Admittedly, response to the adult host was abnormally low but usually such a reduction is paralleled in the response to the young host. Such a reduction was not observed and it is therefore thought to have been masked by CO_2 enhancement. Since a comparable enhancement was not observed in choice tests performed in the wind tunnel olfactometer, it is concluded – as is generally accepted – that CO_2 was functioning as a stimulator and not as a primary close range attractant for Aedes aegypti(L.).

McIver (1968) found cold-trapped mouse odor to be attractive when it was not in competition with the live host. She also suggested that it was the concentration of odor and not the odor itself that influences host preferences. Our odor was concentrated from a dozen adult rats and, although it had a distinct rat-like odor to the investigator it did not increase mosquito attractancy to the young host. Perhaps critical chemicals were missed or were destroyed by the odor collecting process. Another possibility is that repellent concentrations of natural skin-surface compounds may have been reached (Skinner et al., 1965, 1967).

From this study it would appear that no single

factor is responsible for differences in mosquito response to adult and young hosts. As demonstrated in the wind tunnel olfactometer tests, the ability of Aedes aegypti(L.) to consistently respond preferentially to the adult host, regardless of stimulus supplementation to the young host, exemplifies the complexity of its discriminating abilities.

C H A P T E R IV

THE EFFECT OF HOST AGE ON MOSQUITO FECUNDITY

Abstract

Aedes aegypti (L.) feeding on three-day-old laboratory rats took blood meals comparable in weight to those of mosquitoes feeding on adult rats. Egg production on both bloods was similar. Blood meals taken in repeated feedings on a newborn mouse did not decrease in size, as determined by gravimetric and hemoglobinometric techniques. However, reduced egg production resulted from blood meals taken after a mouse had previously been fed upon by fifteen mosquitoes.

Introduction

Numerous studies have dealt with the nutritive requirements for mosquito egg production. Both quantitative (Woke *et al.*, 1956; Colless and Chellapah, 1960; Shelton, 1972; Jalil, 1974) and qualitative (Woke 1937; Greenberg, 1951; Downe and Archer, 1975) blood meal factors have been demonstrated to influence mosquito fecundity. The effect of host species on fecundity has been investigated for a number of mosquito species. Findings have consistently shown reptilian and avian blood more nutritive than mammalian blood

(Bennett, 1970; Stahler and Seeley, 1971; Shelton, 1972; Jalil, 1974). Woke et al. (1956) demonstrated no change in fecundity when conspecific host factors were examined. Host age was not considered in their study. The importance of host age in determining fecundity has been shown for other blood feeding arthropods (Buxton, 1948; DeMeillon and Hardy, 1951).

The present study was designed to determine (1) whether host age affects mosquito fecundity and (2) whether repeated blood-feeding on a single newborn host affects fecundity. Both gravimetric and hemoglobinometric techniques were used to determine blood meal size.

Materials and Methods

Mosquitoes. Aedes aegypti(L.) Georgia strain, which have been continuously maintained in our lab for three years on human blood, were used in all experiments. Larvae were reared in enamel pans at a density of 250 per liter of water per pan. Larval diet consisted of 50% Brewer's yeast and 50% lactalbumin. Adults were maintained on a 2% sucrose solution and were sugar-starved 12 hours before blood feeding. Adults were maintained at 27°C, 75-80% RH with a 14:10 h light:dark cycle (illum. 1076 lux). Female mosquitoes previously housed with males and 5-8 days old were used in experiments.

Host age and fecundity studies. To obtain the mean weight of unfed mosquitoes, fifty mosquitoes were anesthetized on ice and weighed individually on a Mettler H51AR balance. Mosquitoes (150) were allowed to feed to repletion on a restrained adult female laboratory rat. Another 150 were divided into groups of 25 and allowed to feed to repletion on 6 restrained 3-day-old rats. Fifty of the recently fed (within two minutes of completion) mosquitoes were collected from both the adult and young host, anesthetized on ice and weighed individually. The mean weight of the blood meals were calculated from these data. The remaining 100 blood-fed mosquitoes from each host group feeding were maintained for 3 days on 2% sucrose and then dissected under a binocular microscope. Wing length (from thorax to wing tip) was measured with an ocular micrometer and the number of developed eggs recorded.

Repeated blood feeding and fecundity. An adult female laboratory mouse and 6 of her 1-day-old young were restrained and each fed on by 16 mosquitoes in the following manner. Day-old mice were restrained on a glass slide with nylon mesh and were warmed in a human hand. The adult mouse was restrained so that the tail was exposed for feeding. Four mosquitoes were then allowed to feed to repletion on each host. These mosquitoes were designated

the first feeding group and were housed separately according to host and feeding group. Three more feeding groups were similarly obtained so that each host had been fed on sixteen times. Feedings took place in rapid succession. The mosquitoes were then maintained for three days on 2% sucrose and later dissected under a binocular microscope to record the number of eggs developed. Similar experiments were performed using only the day-old young of 3 litters. In these experiments host-feedings were pooled for each feeding group and 5 mosquitoes were used per host per feeding group (5 MHF).

The following experiment was performed to determine the blood meal size during sequential feedings. Six young mice were fed on in the above manner using 4 mosquitoes per host per feeding group (4 MHF). Blood-fed mosquitoes were immediately collected and frozen in separate vials designating their position in the feeding sequence. Fifty mosquitoes of the same test cohort were weighed as before to obtain the mean weight of unfed mosquitoes. Each fed mosquito was weighed and then submitted to a whole abdomen hemoglobinometric analysis as described by Briegel et al. (1979). A blood volume standard series (1 ul, 2 ul, 4 ul and 6 ul) was made using heparinized blood collected from seven decapitated day-old mice. Blood meal weights and optical densities of each feeding lot

were subjected to statistical analysis.

Statistical analysis. The student's t-test was used for data from experiments with 2 treatments. Multiple treatment experiments were subjected to an analysis of variance and, when necessary, a posteriori Student Newman-Keuls test (Sokal and Rohlf, 1969).

Results

Data from the host age and fecundity experiments are summarized in Table 11. In group A, mean blood meal weight and mosquito size were not significantly different between the two treatments. The number of eggs developed on adult host blood was slightly larger and this difference was significant at the 0.05 level. Values for the range of the number of eggs developed and the quality index (eggs/milligram blood) for the two host age treatments were remarkably similar. In contrast significant differences (at the 0.01 level) between treatment values for both mean weight of blood meal and mosquito size were found in group B feedings. Egg production was again slightly larger on adult host blood but this difference was not significant. Interestingly, in group B feedings an average of 15 more eggs was developed on a milligram of young host blood than on adult host blood.

TABLE 11 Number of eggs developed on bloodmeals from adult and three-day-old laboratory rats.

Blood Source	Mean Weight Mosq. Unfed[a] (mg)	Mean Weight Blood Meal[a] (mg)	Mean Mosquito Wing Length[b] (mm)	Number of Eggs Developed X ± SD	Range	Quality Index Eggs/mg Blood
Adult Rat A	2.13	3.07	3.31	138.15 ± 17.22*	55-181	45.00
Young Rat A	2.13	2.93	3.32	132.33 ± 20.17	54-182	45.16
Adult Rat B	1.82	3.24**	3.41	122.89 ± 16.31	82-156	37.93
Young Rat B	1.82	2.23	3.46**	118.91 ± 19.26	50-156	53.32

[a] 50 observations.
[b] 100 observations.
* Significant at 0.05 level.
** Significant at 0.01 level.

Tables 12, 13 and 14 summarize data from repeated blood feeding experiments on individual day-old mice. No significant differences in egg production were found between the individual young hosts (Table 12). Although fewer eggs appeared to be developed on blood meals from the last feeding group (4th), differences between feeding groups were not significant (Table 13). Thirty-eight percent of the mosquitoes in the 4th feeding group were not stimulated to blood feed (Table 13). Significant differences in egg production were found between feeding groups where 5 MHF were used (Table 14). In these instances fewer eggs were developed on blood meals from the 4th feeding group. Also the greatest percentage of zero egg development was from this feeding group.

Finally, findings (Table 15) for the gravimetric and hemaglobinometric analysis on repeated blood feedings indicate there were no significant differences between feeding group for either the mean weights or the optical densities of the blood meals. Blood meal volumes were calculated from the regression of a standard volume series (Figure 10).

Discussion

A number of cellular and molecular blood constituents have been suggested to influence fecundity and some of these are known to change with age. Buxton (1948) and

TABLE 12 Sequential mosquito feedings on individual newborn mice. Egg development on blood from various laboratory mice - adult and newborn young.

Blood Source	N	Number of Blood meals	Percent Not Feeding	Number of Eggs Developed X ± SD	Number of Eggs Developed Range
Adult Mouse (control)	16	16	0	128.33 ± 20.47[a]	91 - 161
Young Mouse A	16	11	31	132.73 ± 17.16[a]	108 - 156
Young Mouse B	16	16	0	108.07 + 36.86[a]	35 - 163
Young Mouse C	16	16	0	117.38 ± 36.49[a]	53 - 156
Young Mouse D	16	16	0	135.31 ± 20.25[a]	95 - 158
Young Mouse E	16	15	6	126.47 ± 30.42[a]	33 - 157
Young Mouse F	16	11	31	133.73 ± 30.05[a]	81 - 178

[a] Means followed by the same letter are not significantly different.

TABLE 13 Sequential mosquito feedings on individual newborn mice. Egg development for various feeding groups - 4 mosquitoes per host per feeding group.

Feeding Group	N	Number of Blood Meals	Percent Not Feeding	Number of Eggs Developed X ± SD	Range
1	24	24	0	131.2 ± 28.7[a]	28 - 165
2	24	24	0	128.7 ± 18.7[a]	97 - 157
3	24	24	0	126.0 ± 29.8[a]	81 - 178
4	24	14	38	113.1 ± 43.0[a]	33 - 158

[a] Means followed by the same letter are not significantly different.

TABLE 14 Sequential mosquito feedings on individual newborn mice. Egg development for various feeding Groups - 5 mosquitoes per host per feeding group.

Litter	Feeding Group	Number of Blood meals	Percent PDBM[1]	Percent ZED[2]	Number of Eggs Developed X ± SD	Range
1	1	48	2	4	104.69 ± 29.93[a]	20 - 160
	2	46	7	4	110.63 ± 32.26[a]	21 - 164
	3	48	2	8	95.65 ± 38.15[a]	5 - 153
	4	33	3	21	98.56 ± 31.19[a]	21 - 144
2	1	39	-	-	136.30 ± 19.85[a]	60 - 174
	2	40	-	-	125.00 ± 26.56[ab]	23 - 157
	3	28	-	-	137.60 ± 21.59[a]	87 - 173
	4	35	-	14	118.73 ± 36.60[b]	19 - 180
3	1	50	4	2	107.57 ± 19.31[a]	66 - 146
	2	44	-	9	101.38 ± 24.37[a]	37 - 146
	3	32	-	9	100.33 ± 22.94[a]	60 - 136
	4	10	-	20	80.75 ± 12.62[b]	62 - 95

[1] PDBM = partially digested blood meal.

[2] ZED = zero egg development.

[a] Means followed by the same letter are not significantly different.

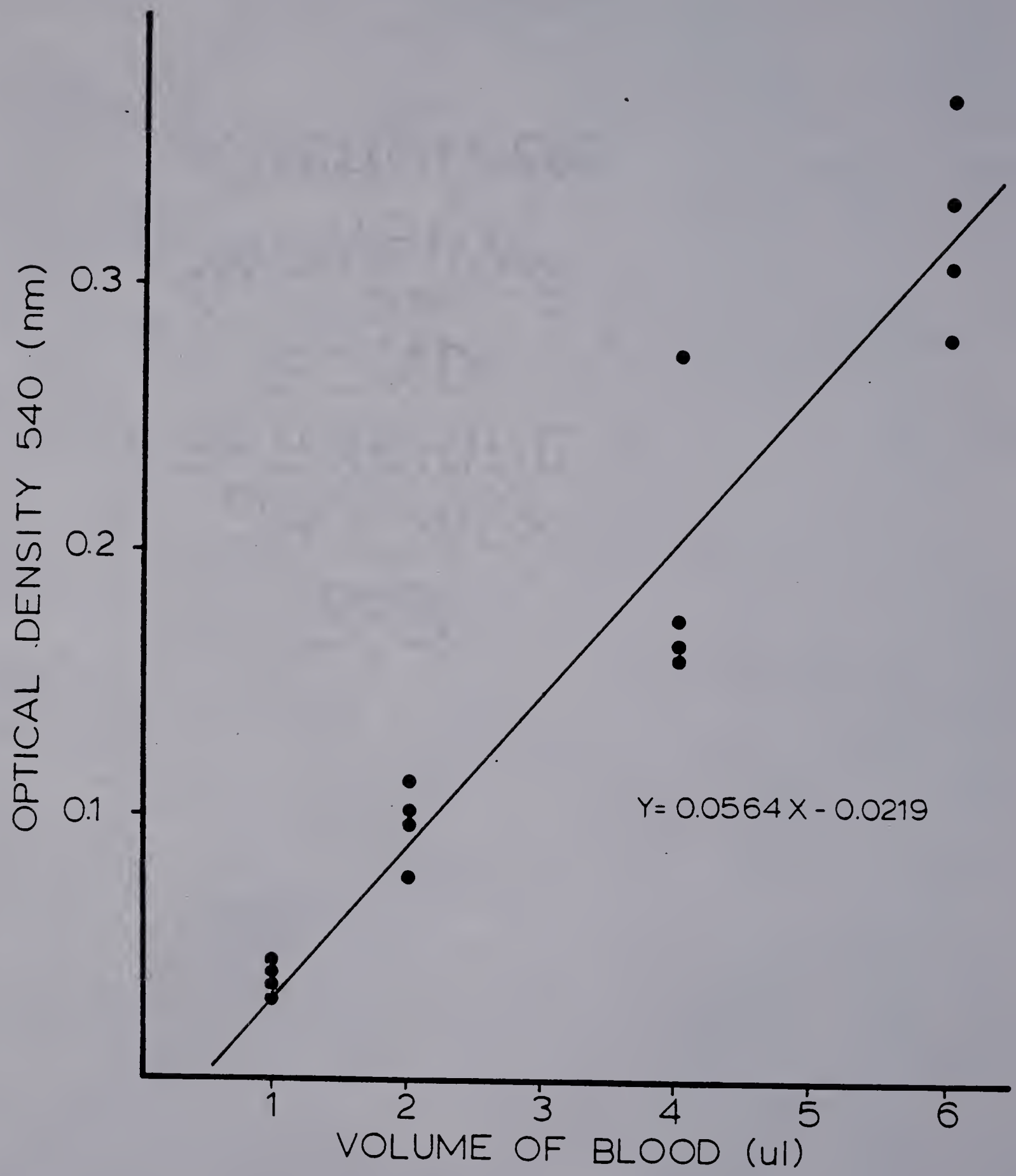
TABLE 15 Sequential mosquito feedings on individual newborn mice. Blood meal weights and volume for various feeding groups. Four mosquitoes per host per feeding group.

Feeding Group	Mean Weight Mosq. Unfed (mg)	Weight (mg) After Feeding X ± SD	Mean Weight Blood Meal (mg)	Optical Density (540 mm) X ± SD	Mean Volume Blood Meal[1] (ul)
1	2.10	4.67 ± 0.99 [a]	2.57	0.1596 ± 0.0872 [a]	3.215
2	2.10	4.51 ± 0.85 [a]	2.41	0.1519 ± 0.0844 [a]	3.079
3	2.10	4.80 ± 0.88 [a]	2.70	0.1923 ± 0.0819 [a]	3.795
4	2.10	4.55 ± 0.73 [a]	2.45	0.1433 ± 0.0734 [a]	2.926

[1] Calculated from standard curve, Y = 0.05644X - 0.02187.

[a] Means followed by the same letter are not significantly different.

Figure 10. Linear regression of HiCN (OD_{540}) and the amount of blood from suckling rats added to Drabkin's reagent. Standard curve.


OPTICAL DENSITY 540 (nm)
0.3
0.2
0.1
Y= 0.0564 X - 0.0219
1
2
3
4
5
6
VOLUME OF BLOOD (ul)

DeMeillon and Hardy (1951) suggested that sex hormone levels and hemolysis of fetal erythrocytes, respectively, may have contributed to reduced fecundity in hematophagous insects fed on young hosts. The association between nucleated erthyocytes and fecundity is well documented (Woke, 1937; Bermett, 1970; Downe and Archer, 1975) and the importance of amino acids, especially isoleucine, has been demonstrated by Lea et al. (1956, 1958) and Chang and Judson (1977, 1979). Both amino acid levels and erthyocytes are known to change with age in rats (Creskoff and Fitz-Hugh, 1942, Munro, 1970).

No evidence was found to suggest that mosquito fecundity was appreciably affected by host age. In both replications more eggs were developed on adult rat blood but only in one of the replications was this difference significant. Between treatment variation in the values of mosquito size and blood meal weight made interpretation of group B results difficult. Disparity in the quality indices might have been due to significant differences in mosquito size although it is more likely that 2.23 mg of blood was within the optimal range for egg production (Colless and Challapah, 1960; Shelton, 1972; Jalil, 1974). Since the quality indices for group A were essentially identical, it is unlikely that age-associated blood factors were influencing fecundity.

Repeated blood feeding on newborn mice did affect mosquito fecundity. In tests using 5 MHF the last feeding group was characterized by the highest percentage of zero egg development. Observed 'blood'-feedings that were complete but did not result in egg development may have contained increased values of other, nonnutritive, body fluids. The vulnerable state of a newborn mouse makes deep tissues and organs accessable to the mosquito during feeding. Also, in two of the three tests with 5 MHF, there was a significant reduction in fecundity observed in the last feeding. The lack of difference between feeding groups in a test using 4 MHF may indicate a threshold number of feedings before fecundity is affected. According to the data this would be between 15 and 20 complete blood meals taken on a single host.

Gravimetric measurement showed that there was no reduction in the blood meal size of succeeding feedings. Similarly hemoglobinometric analysis confirmed the stability of blood meal size thoughout feedings and also negated the possibility of feeding-induced anemia. However, these measurements were made using 4 MHF and differences in fecundity were found only in 5 MHF tests. Blood meal size reduction may not be gradual and therefore remain undetected in these measurements. Traumatic blood loss occurring at the threshold number of feedings may

shift blood into deeper tissue. Alternatively some unmeasured factor was responsible for reduced fecundity. Perhaps chemicals associated with the host's inflamatory response interfer with egg production.

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